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Original Research Paper

Effects of Hydroalcoholic Extract of *Dorema aucheri* on Pituitary-Gonad Axis Hormones in Adult Male Rats

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ABSTRACT

Dorema aucheri is a plant from Apiaceae family, which has several compounds such as flavonoids and kumarines. Flavonoids have antitumor, anticancer and estrogenic properties. Antiandrogenic properties of kumarines are also known. The aim of this research is to examine the hydroalcoholic extract of *Dorema aucheri* on serum concentration of main pituitary-gonad axis hormones including LH, FSH, testosterone and dihydrotestosterone. Fifty adult male wistar rats weighting about 200 to 220 g were divided into five groups of ten. The control group did not receive any drug and sham operation group received an equal volume of normal saline. Other three experimental groups including low (100 mg/kg bw), medium (200 mg/kg bw) and maximum (400 mg/kg bw) received *Dorema aucheri* hydroalcoholic extract daily for 28 days orally. After 28 days all animals in the different groups were weighed and after anaesthetizing with ether, their blood was collected and serum concentrations of hormones LH, FSH, testosterone and dihydrotestosterone were measured by ELISA method. Data were statistically analysed by ANOVA and t-test. The results showed that LH concentrations in all experimental groups significantly increase in comparison to control and sham groups, while FSH concentrations among the different groups did not change significantly. The concentrations of testosterone and DHT in different experimental groups decreased significantly in comparison to control and sham groups. The results of the study indicate that hydroalcoholic extract of *Dorema aucheri,* due to estrogenic and antiandrogenic properties of flavonoids and kumarines, can change serum concentrations of pituitary-gonad axis hormones.

INTRODUCTION

The plant *Dorema aucheri* with local name bilhar or kandal grows in the central region of Iran and in border of Zagros Mountains. This plant belongs to Family Apiaceae with hollow stem and leaves alternating with large divisions and has umbelliferous inflorescence. This plant has bisexual flowers and achene fruits. All plant organs have secretary activity (Gahreman 1996). For the first time flavonoids and kumarines were extracted from *Dorema aucheri* (Wollen Weber 1995). Flavonoids are the members of active chemical compounds named phytoestrogens (Pangeshahin et al. 2005). They have significant antioxidant, anti-allergy, anti-inflammatory and anticancer activity (Farsam 2000). In addition to its kumarines extract, the plant has very potent anti-aromatase, anti-androgenic and estrogenic activity (Mirzaee 2005). Since, this plant is abundant used for food consumption, two main aims for the present research were taken into consideration, first, to determine possible effect of its hydroalcoholic extract consumption on pituitary-gonad axis activity, and second, to offer advice to consumers of this plant regarding its benefits and risks of consumption. Consequently, the possible changes in its hormone concentrations including LH, FSH, testosterone, dihydrotestosterone can

be reviewed and possible results will be used by scientific and research community to extrapolate them to humans.

MATERIALS AND METHODS

The animals used in this study were 50 wistar adult male rats. Their approximate weight and age were 200 to 220 g and 2.5 to 3 months respectively. The Animals were taken from animal house of Yasuj Medical Science University and maintained in stable temperature condition of 23±3°C in separate polycarbonate cages.

Light period was adjusted 12 hours light (from 07.00 a.m. to 07.00 p.m.) and 12 hours darkness (from 07.00 p.m. to 07.00 a.m.) respectively. Water and food were provided without any restriction to all the animals. For adaptation with experimental condition, the research was started a week after that animals kept in this situation. The animals were divided into 5 groups of 10. The control group did not receive any drug or experimental treatment. The sham operated group received daily 2 mL distilled water orally. Three experimental groups: (1) low dose group that daily received at least 100 mg/kg bw *Dorema aucheri* hydroalcoholic extract, (2) medium dose group that received daily at least 200 mg/kg bw *Dorema aucheri* hydroalcoholic extract, and (3) maximum dose group that received daily 400 mg/kg bw *Dorema aucheri* hydroalcoholic extract. Experimental period for all groups was 28 days. After the 28-day period, all animals were weighed and then were killed and blood samples were collected directly from their heart. Animal serum samples were isolated from blood and serum hormones. LH, FSH, testosterone, dihydrotestosterone were measured by ELISA method. Right and left testes of the animals were taken out and after cleaning they were weighed. The results based on statistical methods and tests of ANOVA and t-test were analysed. Significant level of $P \leq 0.5$ was considered.

RESULTS

As shown in Table 1, there were no significant differences in mean body weight among all the experimental and control groups. Moreover, mean weight of left and right testes in all groups does not show any significant difference (Table 2). Based on the results obtained and shown in Table 3, the mean serum LH concentration in all the experimental groups increased significantly compared with control and sham groups ($p \leq 0.05$).

Changes in mean concentrations of LH in different experimental groups compared with each other are not significant. Mean serum FSH concentration in different experimental groups compared with each other and with control and sham groups does not show significant changes. Table 4 shows that there is a significant increase in mean serum concentration of testosterone in low dose group compared with control and sham groups ($p \leq 0.01$).

Mean serum concentration of testosterone in medium and maximum dose groups decreased significantly in comparison with control and sham groups ($p \leq 0.05$). In comparison with the concentrations of testosterone in experimental groups to each other, the results show that mean serum concentration of testosterone in medium and maximum dose groups significantly decrease to low dose group ($p \leq 0.01$). These results indicate that the responses of testosterone to administered different amounts of *Dorema aucheri* hydroalcoholic extracts are different. The study also determined that the mean serum concentration of dihydrotestosterone in all the experimental groups decrease significantly compared with control and sham groups ($p \leq 0.05$), while in different experimental groups compared with each other, there is no significant difference.

Table 1: The effects of *Dorema aucheri* hydroalcoholic extract on body weight in different groups.

Groups	Mean body weight (g)($\overline{x}$ ± SEM)
Control	255 ± 33.69
Sham	264.2 ± 22.09
Low dose group (100 mg/kg bw)	256.4 ± 10.4
Medium dose group (200 mg/kg bw)	259 ± 16.7
Maximum dose group (400 mg/kg bw)	267 ± 19.3

Table 2: The effects of *Dorema aucheri* hydroalcoholic extract on right and left testes weight in different groups.

Groups	Mean right testis weight (g) ($\overline{x}$ ± SEM)	Mean left testis weight (g) ($\overline{x}$ ± SEM)
Control	1.27 ± 0.11	1.35 ± 0.19
Sham	1.30 ± 0.17	1.32 ± 0.23
Low dose group (100 mg/kg bw)	1.28 ± 0.86	1.4 ± 0.05
Medium dose group (200 mg/kg bw)	1.28 ± 0.07	1.26 ± 0.05
Maximum dose group (400 mg/kg bw)	1.32 ± 0.15	1.34 ± 0.15

Table 3: The effects of *Dorema aucheri* hydroalcoholic extract on serum concentration of LH and FSH in different groups.

Groups	Mean concentration of LH (Iu/L) ($\overline{x}$ ± SEM)	Mean concentration of FSH(Iu/L) ($\overline{x}$ ± SEM)
Control	5.99 ± 3.07	1.76 ± 0.03
Sham	6.59 ± 2.6	1.75 ± 0.08
Low dose group (100 mg/kg bw)	9.19 ± 0.52*	1.75 ± 0.05
Medium dose group (200 mg/kg bw)	1.83 ± 0.46*	1.83 ± 0.2
Maximum dose group (400 mg/kg bw)	1.7 ± 0.32*	1.7 ± 0.04

The mean amounts marked by (*) have a significant difference compared with control and sham groups ($P \leq 0.05$).

Table 4: The effects of *Dorema aucheri* hydroalcoholic extract on serum concentration of testosterone and dihydrotestosterone in different groups.

Groups	Mean concentration of testosterone (Iu/L) ($\overline{x}$ ± SEM)	Mean concentration of DHT (ng/L) ($\overline{x}$ ± SEM)
Control	3.33 ± 0.27	31.58 ± 3.8
Sham	3.21 ± 0.20	31.88 ± 3.2
Low dose group (100 mg/kg bw)	7.21 ± 0.59**	23.96 ± 2.5 *
Medium dose group (200 mg/kg bw)	1.53 ± 0.87 *	27.29 ± 1.4 *
Maximum dose group (400 mg/kg bw)	1.61 ±0.10 *	27.27 ±2.3 *

The mean amounts marked by (*) and (**) have significant differences compared with control and sham groups (*$P \leq 0.05$) (** $P \leq 0.01$)

DISCUSSION

In hydroalcoholic extract of *Dorema aucheri* there is considerable amount of flavonoid compounds, and this suggests that administration of this extract can cause significant reduction in body weight in all experimental groups compared with control and sham groups, because flavonoids inhibit esterase

enzyme, phosphodiesterase (PDE) and, therefore, increase lipolysis in adipose tissues (Pleuso 2006, Giovanni et al. 2007). On the other hand flavonoids inhibit activity of 3-hydroxy glutaryl co enzyme A (HMG-CoA) enzyme. This enzyme is the key enzyme in cholesterol biosynthesis in liver and thus, these properties can reduce body weight significantly (Middleton et al. 2000). But the results obtained in this study do not show significant reduction in body weight of animals in experimental groups. Perhaps because of short period of hydroalcoholic extract of *Dorema aucheri* administration in experimental animals, there is not sufficient time to influence the metabolism of lipids by flavonoids. Spencer et al. (2007) showed that some types of flavonoids connect to ATP binding site in ATP-sensitive enzymes and then change specific activity of this enzymes or affect their cell membrane receptors in different cells. This mechanism shows non-selective and wide effects of flavonoids on adipose tissues. There are many different reasons for ineffectiveness of administration of hydroalcoholic extract of *Dorema aucheri* on testes weight in the experimental groups. For example, very complex mechanisms control testes size and weight; in addition most of the mechanisms differ from common mechanisms that control growth in other tissues. In addition, common trophic hormone, for example, growth hormone (GH) cannot modulate growth and development in many cells and parts of testes. Pituitary-gonad axis hormones such as FSH and LH control growth process in testes. In this study, because of significant changes in FSH and LH concentration in experimental groups, changes in testes weight were expected. But this result could not be shown due to short period of the study. Significant increase in concentration of LH hormone in experimental groups compared with control and sham groups has several reasons. Flavonoids in *Dorema aucheri* hydroalcoholic extract have phytoestrogenic properties (Formica & Regelson 1995). Estrogen can stimulate synthesis and secretion of prolactin and these hormones have direct effects on gonadotropins secretion, including LH response to GnRH (Bowen 2003). On the other hand Seong et al. (1995) and Wanger Edward et al. (2001) showed that GABAergic neurons in preoptic area of hypothalamus through negative feedback reduce the production and secretion of LH, and estrogen is capable of inhibiting these hypothalamic GABAergic neurons and thus, eliminating the negative feedback inhibitory mechanism can increase serum LH concentration. Based on the results, serum concentration of testosterone increased significantly in low dose experimental group compared with control and sham groups. This increase may be is due to increase in serum LH concentration in this group. On the other hand, it is possible that flavonoid compounds in *Dorema aucheri* hydroalcoholic extract inhibit testosterone metabolism enzymes like aromatase and 5-alpha reductase and, therefore, increase serum testosterone concentration (Papies 2004). Also flavonoid compounds in *Dorema aucheri* hydroalcoholic extract have both estrogenic and non-estrogenic properties (Miksisek 1999). Since phytostrogens act as estrogen synthesis inhibitor via two mechanism; 1. blocking aromatase enzyme function and reducing converting testosterone to estrogen, and 2. competitively bind to estrogen receptors; they can increase serum concentration of testosterone (Wang et al. 1994). The results show a significant decrease in serum concentration of testosterone in medium and maximum dose groups compared with control and sham groups. Sadeghi et al. (2004) showed that administration of *Dorema aucheri* extract in high doses reduce cholesterol synthesis. In present study, it seems that administration of high doses of *Dorema aucheri* hydroalcoholic extract in experimental groups can induce significant decrease in serum concentration of testosterone by comarines, because comarines in high concentration have antiandrogenic properties (Shiuan et al. 2004). Mean serum concentrations of dihydrotestosterone have significant decrease in experimental groups compared with control and sham groups, probably due to the presence of flavonoids compounds, because flavonoids can inhibit 5 alpha reductase enzyme activities, which converts testosterone to dihydrotestosterone (Papies 2004).

On the other hand, phytostrogens in *Dorema aucheri* hydroalcoholic extract stimulate SHBG synthesis in liver, after the increase in SHBG concentration, decreased serum dihydrotestosterone is predictable. The results show that there are no significant differences in serum concentration of FSH between experimental groups and control and sham groups. It may be due to modulate effects of important pituitary-gonad axis hormones such as inhibin, activin and folestatin. In addition to feedback mechanisms by which testicular steroids are applied, these compounds may affect the amount of GnRH synthesis by central mechanism and, therefore, have important role in modulation of FSH concentration. Another possible mechanism is that the metabolic clearance of FSH is slower than LH and that is why, changes in serum concentration of FSH are slower than LH (Araki et al. 2000).

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